THE POTENTIAL OF MYCORRHIZAL TECHNOLOGY IN IMPROVING THE PRODUCTION OF PLANT BIOACTIVE COMPOUNDS:

What is the overview of studies conducted in Brazil?



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Fábio Sérgio Barbosa da Silva
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PREFACE

To read, study or research about arbuscular mycorrhizal fungi (AMF) is often the certainty of finding something new about the theme due to the fundamental role of these fungi in nature and plant development.

They are microorganisms that benefit different botanical families, including plants of economic interest to the food, pharmaceutical, cosmetic, and herbal medicine sectors, among others. Despite the many reports on AMF in scientific papers, it is worth mentioning some of the benefits that these microorganisms provide, such as improving plant nutritional uptake, greater tolerance to environmental stresses and phytopathogens, promoting plant growth, improving the photosynthetic rate and the biosynthesis of compounds like carbohydrates, phenolics, and terpenes. Furthermore, AMF can improve the soil structure, increasing, its water retention capacity.

Thus, having a book that assists the academic community and the population in discovering this AMF 'world', to improve knowledge about these microorganisms and stimulate the development of research, is paramount for scientific and economic growth. With this in mind, this book compiles work carried out in Brazil showing the research development about the production of bioactive compounds in AMF-associated plants in the Brazilian regions. For example, the reader will see how the Northeast region has grown in recent years in terms of studies with these fungi. In contrast, the North region has less work on the subject but great potential to increase research into the use of AMF. Moreover, it provides possible perspectives and ways forward for studies on the phytochemistry of mycorrhizal species in Brazil, given the country's biodiversity and the potential for research on this subject.

You, the reader, will find a book that is written in a way that aids comprehension, with figures and graphs that will help you to read and understand AMF and with a chapter organization that will make it easier for you to see how this research field is being improved here in Brazil. So, I hope it awakens you to a fascination for the vast 'world' of AMF in plant anabolism.

Happy reading!

Emanuela Lima dos Santos

PRESENTATION

Agriculture in Brazil accounts for most of the country's Gross Domestic Product (GDP), providing raw materials for the food, medicine, and cosmetics markets, among others. Some strategies can increase crop productivity, such as inoculating arbuscular mycorrhizal fungi (AMF), an efficient tool for optimizing the biosynthesis of bioactive compounds in several plants. For this reason, this book brings together research papers carried out for over two decades in Brazil to evaluate the production of secondary metabolites in mycorrhizal plants, especially those of medicinal importance. In this approach, the botanical families, plant organs, AMF species, and compound groups evaluated over these years of studies have been listed, taking into consideration the main Brazilian research groups working in this area. The chapters were also produced to encourage the integration of researchers working in this area of mycorrhizology in Brazil, highlighting some gaps that could be investigated.

We wish you an enriching read!

Fábio Sérgio Barbosa da Silva
Eduarda Lins Falcão
Organizers

SUMMARY

CHAPTER 1

OVERVIEW OF BRAZILIAN STUDIES ON PHYTOCHEMISTRY OF MYCORRHIZAL SPECIES

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ABSTRACT: Mycorrhizal technology to enhance the production of plant bioactive compounds in Brazil has been studied for around 20 years and has given promising results for the Brazilian industry. Therefore, this review aimed to present the research on the phytochemistry of species inoculated with arbuscular mycorrhizal fungi (AMF) in Brazil, to assist research groups in selecting isolates that are effective in boosting the production of bioactive compounds. Based on database searches (Web of Science

and National Center for Biotechnology Information), 66 experimental papers, four reviews, one editorial, and one opinion paper were selected. An overview of AMF species, botanical families, regions where studies have been carried out, experimental methods, main groups of biomolecules, and the most evaluated mycorrhizal parameters in the country were summarized. It was observed that Northeast Brazil accounts for more than 50% of all studies on the phytochemical aspect of mycorrhizal plants. The isolates of *Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno, *Acaulospora longula* Spain & N.C. Schenck, and *Gigaspora albida* N.C. Schenck & G.S. Sm. are the most tested in phytochemical studies of mycorrhizal species in the country, with results mainly reported under greenhouse conditions, as only six studies have been carried out under field conditions. The application of AMF can potentially increase the production of secondary compounds in plants, especially in *Fabaceae* representatives, which occur in Brazil, becoming an agronomic tool for the Brazilian pharmaceutical and cosmetic industries.
KEYWORDS: *Entrophospora*, mycorrhizal fungi, *Glomeromycota*, secondary metabolites.

1. INTRODUCTION

The supply of raw materials from plants is essential to meet the global demand for food and medicine (Maroyi, 2022). From this perspective, Brazil has a high potential as the country with the world's greatest biodiversity, due to its vast plant genetic heritage (Brasil, 2016), including medicinal resources for the industry.

Raw plant materials can be used to formulate medicines due to the presence of pharmacologically active compounds (Bernardes *et al.*, 2017). Examples of these are products marketed by pharmaceutical companies, such as coumarins obtained from *Mikania laevigata* Sch. Bip. ex Baker, vitexin found in *Passiflora alata* Curtis, senosides A and B produced by *Senna alexandrina* Mill. and valerenic acid, extracted from *Valeriana officinalis* L. (ANVISA, 2019). These compounds, among others found in products of plant origin, contributed to a revenue of more than R$300 million in Brazil in 2019 (ANVISA, 2021).

In addition to herbal medicines, cosmetic products with moisturizing, depigmenting, anti-acne, repairing, and sun protection properties can also contain plant-derived ingredients. In such products, species like *Aloe vera* (L.) Burm. f. (Nivea®) (www.niveausa.com), *Melaleuca alternifolia* Cheel (Sallve®) (www.sallve.com.br), *Agathosma betulina* (Bergius) Pillans (www.sallve.com.br), *Rosa canina* L. (Sallve®) (www. sallve.com.br), *Bidens pilosa* L. (Sallve®) (www.sallve.com.br), *Centella asiatica* L. (La Roche-Posay®) (www.laroche-posay.pt), and *Theobroma cacao* L. (Natura®) (www.natura.com.br) are used by national and international companies. However, it is important to improve the quality of the raw materials used to manufacture these and other products, as they can vary in metabolite content (Barbosa *et al.*, 2008).

Among the agro-biotechnologies available to improve plant production, beneficial microorganisms are promising, especially those that form mutualistic associations, such as arbuscular mycorrhizal fungi (AMF). This biotechnology has been tested to promote

the biosynthesis of secondary plant metabolites in Brazil for over 20 years (Freitas *et al.*, 2004a). It generates yields that exceed 500% to produce pharmaceutically and cosmetically relevant phytochemicals (Falcão *et al.*, 2022).

AMF are obligate biotrophs (Redecker *et al.*, 2013), belonging to the phylum *Glomeromycota* and classified into 17 families and 50 genera (Wijayawardene *et al.*, 2022). In Brazil, 38 AMF genera are present in national biomes (Maia *et al.*, 2020), with most species belonging to *Glomeraceae* and *Acaulosporaceae* (Maia *et al.*, 2020). These fungi form a symbiotic association from the emission of the germ tube (Tanaka *et al.*, 2022), an asymbiotic hypha that comes into contact with the root (Hepper, 1985) and differentiates into an appressorium (Mosse; Hepper, 1975). After penetration, the hyphae grow through the root cortex into the intercellular (Cox; Sanders, 1974; Mosse; Hepper, 1975) and intracellular spaces, where arbuscules are formed; in these, nutrient exchange takes place between the fungus and the host (Cox; Sanders, 1974; Marx *et al.*, 1982). An external mycelium is formed after establishing intracellular root colonization, which commonly restarts the life cycle, producing new glomerospores (Mosse; Hepper, 1975).

To apply these fungi, it is recommended to produce considerable amounts of AMF inoculum containing spores, hyphae, and fragments of colonized roots. They are obtained through substrate cultivation (Silva *et al.*, 2014a; Selvakumar *et al.*, 2016;2018a), which can be by monosporic culture (Selvakumar *et al.*, 2018b), transformed roots (Srinivasan *et al.*, 2014), in aeroponic (Mohammad *et al.*, 2000) or hydroponic systems (Nurbaity *et al.*, 2019).

The cost of producing soil-inoculum is relatively low and can range from 0.02 - 1.30 USD per pot (Santana *et al.*, 2014; Silva; Silva, 2020). However, this technology has not been commercialized in Brazil yet. Considering the diversity of AMF representatives in Brazilian soils (Maia *et al.*, 2020) with recognized efficiency (Pedone Bonfim *et al.*, 2015; Falcão; Silva, 2023), the use of these microorganisms should be encouraged without sticking only to isolates marketed abroad (Basiru *et al.*, 2021). When AMF propagules are applied (Muniz *et al.*, 2021), the fungi benefits to the host plant can be identified (Chen *et al.*, 2017; Mathur *et al.*, 2018); among these, the enhanced production of metabolites stands out, which can be explained by nutritional, physiological and molecular modulations in the photobiont, as summarized in Figure 1.

The benefits of applying AMF in the production of bioactive compounds can be numerous (Wu *et al.*, 2023; Falcão *et al.,* 2023a), considering studies conducted in Brazil. Notwithstanding, compiled data on such symbiotic efficiency are not available, even though comprehensive reviews have been published worldwide (Pedone Bonfim *et al.*, 2015; Sharma *et al.*, 2017; Kaur; Suseela, 2020; Zhao *et al.*, 2022; Thokchom *et al.*, 2023; Falcão; Silva, 2023; Falcão *et al.,* 2024a). Therefore, this review aimed to compile papers on the phytochemistry of mycorrhizal plants from studies conducted in Brazil.

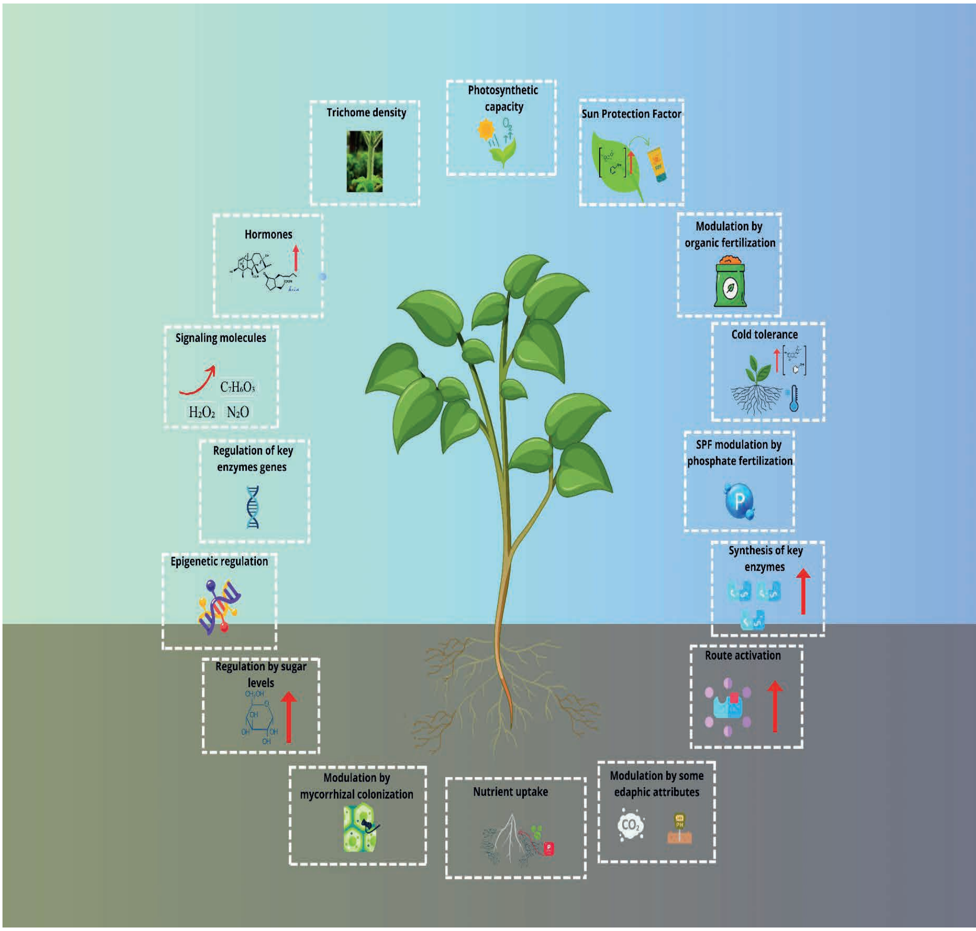


Figure 1. Mechanisms that explain the improved biosynthesis of secondary compounds in response to mycorrhization (Lohse *et al*., 2005; Kapoor *et al*., 2007; Zubek *et al*., 2010; Mandal *et al*., 2013; Zhang *et al*., 2013; Torres *et al*., 2015; Sharma *et al*., 2017; Cui *et al*., 2019; Ran *et al*., 2021; Cela *et al*., 2022; Falcão *et al*., 2022;2023b;2024b; Muniz *et al*., 2023).
Icons: canva.com

2. MATERIAL AND METHODS

A descriptive review was conducted using combinations of descriptors related to studies on AMF and phytochemistry, with terms in English and research time interval of 22 years (2002 to June 2024), as shown in Figure 2. In total, 433 articles were found, considering the search on the National Center for Biotechnology Information (NCBI) and Web of Science platforms, disregarding those repeated in both databases. After initial screening of titles, abstracts, keywords, and methodology, the aims of the papers were also assessed so that only those that focused on increasing the production of biomolecules with mycorrhizal inoculation and were setup in Brazil were included in this review. Thus, 72 papers were selected (Figure 2).

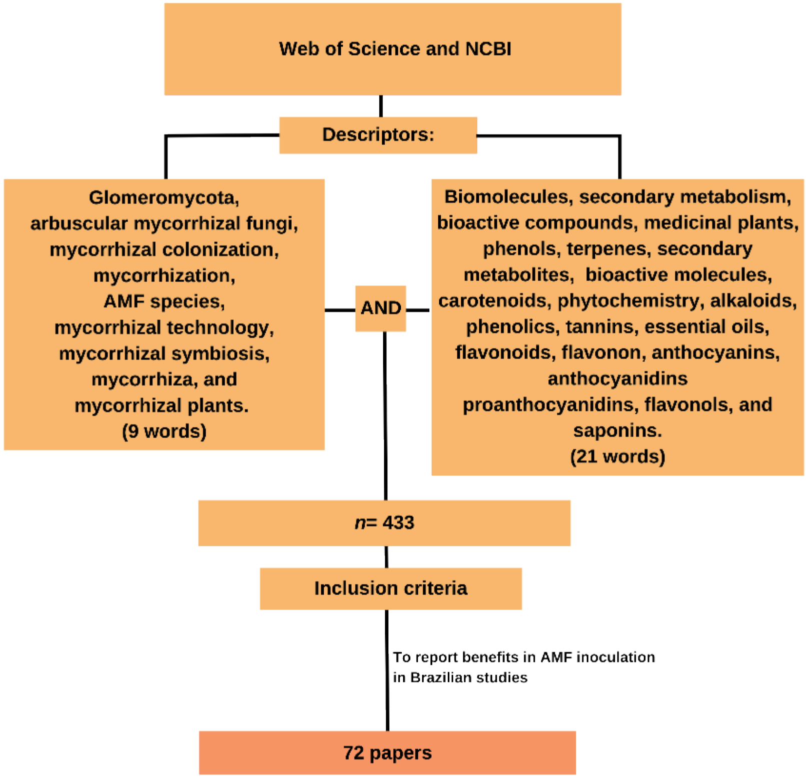


Figure 2. Flowchart of the search based on descriptors related to studies of arbuscular mycorrhizal fungi and the evaluation of the phytochemistry of inoculated plants whose research was conducted in Brazil. NCBI= National Center for Biotechnology Information.

The data from the selected papers were quantified, and the results were expressed as percentages and plotted on graphs. However, of these 72 papers, four were review papers (Pedone Bonfim *et al.*, 2015; Santos *et al.*, 2021a; Falcão; Silva, 2023; Falcão *et al.*, 2024a), one opinion paper (Falcão *et al.,* 2023a), and one was published as an editorial (Wu *et al.*, 2023) so they were not included in the counting presented. In addition, a map was built to plot the distribution of all studies by region and Brazilian states, using Canva (canva.com) (see chapter 2).

3. RESULTS AND DISCUSSION: OVERVIEW OF BRAZILIAN STUDIES ON THE PHYTOCHEMISTRY OF MYCORRHIZAL SPECIES

In Brazil, the main AMF isolates used to increase the production of plant bioactive compounds were *Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno (previously classified as *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler or *Glomus etunicatum* W.N. Becker & Gerd.), *Acaulospora longula Spain* & N.C. Schenck (also considered *Acaulospora morrowiae* Spain & N.C. Schenck),

Gigaspora albida N.C. Schenck & G.S. Sm. and *Rhizoglomus clarum* (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl (previously classified as *Rhizophagus clarus* (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüßler or *Glomus clarus* T.H. Nicolson & N.C. Schenck) (Figure 3). This pattern was partially observed in the review by Zhao *et al.* (2022), which systematized studies conducted worldwide.

Other species evaluated were *Acaulospora colombiana* (Spain & N.C. Schenck) Kaonongbua, J.B. Morton & Bever (previously classified as *Entrophospora colombiana* Spain & N.C. Schenck), *Acaulospora koskei* Błaszk, *Acaulospora scrobiculata* Trappe, *Dentiscutata heterogama* (T.H. Nicolson & Gerd.) Sieverd, F.A. Souza & Oehl [previously classified as *Scutellospora heterogama* (Nicol. & Gerd.) Sieverd., Souza & Oehl], *Diversispora versiformis* (P. Karst.) Oehl, G.A. Silva & Sieverd. [previously classified as *Glomus versiforme* (P.Karst.) S.M. Berch], *Entrophospora claroidea* (N.C. Schenck & G.S. Sm.) Błaszk., Niezgoda, B.T. Goto & Magurno, *Funneliformis geosporum* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *Fuscutata heterogama* Oehl, F.A. Souza, L.C. Maia & Sieverd., *Gigaspora decipiens* I.R. Hall & L.K. Abbott, *Gigaspora margarita* W.N. Becker & I.R. Hall, *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl [previously classified as *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.)] C. Walker & A. Schüßler or *Glomus intraradices* N.C. Schenck & G.S. Sm.), *Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl [also known as *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler], *Cetraspora pellucida* (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd., *Acaulospora mellea* Spain & N.C. Schenck, *Septoglomus viscosum* (T.H. Nicolson) C. Walker, D. Redecker, Stiller & A. Schüßler, and *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders (Figure 3).

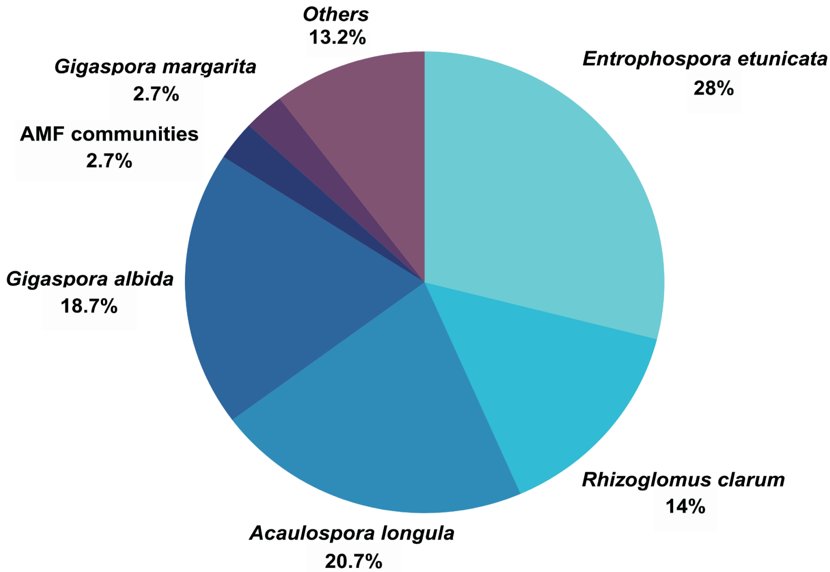


Figure 3. Tested AMF species in studies carried out in Brazil using arbuscular mycorrhizal fungi (AMF) to increase the production of phytochemicals. Number of experimental studies= 66. *Acaulospora colombiana* (Spain & N.C. Schenck) Kaonongbua, J.B. Morton & Bever, *Acaulospora koskei* Błaszk., *Acaulospora longula* Spain & N.C. Schenck, *Acaulospora scrobiculata* Trappe, *Dentiscutata heterogama* (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl, *Diversispora versiformis* (P. Karst.) Oehl, G.A. Silva & Sieverd., *Entrophospora claroidea* (N.C. Schenck & G.S. Sm.) Błaszk., Niezgoda, B.T. Goto & Magurno, *Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno, *Funneliformis geosporum* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *Fuscutata heterogama* Oehl, F.A. Souza, L.C. Maia & Sieverd., *Gigaspora albida* N.C. Schenck & G.S. Sm., *Gigaspora decipiens* I.R. Hall & L.K. Abbott, *Gigaspora margarita* W.N. Becker & I.R. Hall, *Rhizoglomus clarum* (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl, *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl, *Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl, *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders, *Cetraspora pellucida* (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd., *Acaulospora mellea* Spain & N.C. Schenck, *Septoglomus viscosum* (T.H. Nicolson) C. Walker, D. Redecker, Stiller & A. Schüßler (Freitas *et al.,* 2004a,b; Andrade *et al.*, 2010;2013; Oliveira *et al.*, 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al.*, 2013;2018; Riter Netto *et al.*, 2014; Silva *et al.*, 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al.*, 2015;2023; Lima *et al.*, 2015a,b;2017; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Santos *et al.*, 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al.*, 2018;2020; Silva; Maia, 2018; Chiomento *et al.*, 2019;2021;2022; Cordeiro *et al.*, 2019; Cruz *et al.*, 2019;2020; Ferrari *et al.*, 2020; Merlin *et al.*, 2020; Vieira *et al.*, 2021; Trindade *et al.*, 2021; Muniz *et al.*, 2021;2022a,b;2023; Marcolino *et al.*, 2021; Falcão; Silva, 2022; Falcão *et al.*, 2022;2023b;2024b; Palhares Neto *et al.*, 2022; Pinc *et al.*, 2022; Souza *et al.*, 2022; Luz *et al.*, 2023; Nardi *et al.*, 2024; Melato *et al.,* 2024).

When the distribution of studies by region was considered, *A. longula* and *G. albida,* which often promote plant anabolism, were the most applied fungi in research conducted in Northeast Brazil, region with the highest number of published papers (Figure 4) (Oliveira *et al.*, 2013; Pedone Bonfim *et al.*, 2013;2018; Lima *et al.*, 2015a,2017; Silva *et al.*, 2014a,b,c,d;2018;2019;2021a; Oliveira *et al.*, 2015a,b,c;2019a;2020; Santos *et al.*, 2017;2020;2021b; Silva; Silva, 2017;2020; Silva; Maia, 2018; Muniz *et al.*, 2021;2022a,b;2023; Marcolino *et al.*, 2021; Falcão; Silva, 2022; Falcão *et al.*, 2022;2023b;2024b; Luz *et al.*, 2023).

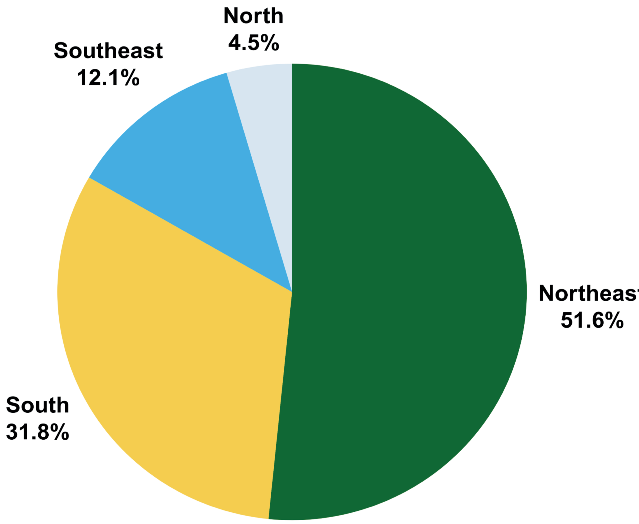


Figure 4. Studies conducted in Brazil that investigated the use of arbuscular mycorrhizal fungi to increase the production of phytochemicals (Freitas *et al.,* 2004a,b; Andrade *et al.*, 2010;2013; Oliveira *et al.*, 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al.*, 2013;2018; Riter Netto *et al.*, 2014; Silva *et al.*, 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al.*, 2015;2023; Lima *et al.*, 2015a,b;2017; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Santos *et al.*, 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al.*, 2018;2020; Silva; Maia, 2018; Chiomento *et al.*, 2019;2021;2022; Cordeiro *et al.*, 2019; Cruz *et al.*, 2019;2020; Ferrari *et al.*, 2020; Merlin *et al.*, 2020; Vieira *et al.*, 2021; Trindade *et al.*, 2021; Muniz *et al.*, 2021;2022a,b;2023; Marcolino *et al.*, 2021; Falcão; Silva, 2022; Falcão *et al.*, 2022;2023b;2024b; Palhares Neto *et al.*, 2022; Pinc *et al.*, 2022; Souza *et al.*, 2022; Luz *et al.*, 2023; Nardi *et al.*, 2024; Melato *et al.,* 2024).

Among the most evaluated botanical families, representatives of *Fabaceae*, *Passifloraceae*, and *Lamiacaeae* were the most tested for the quantification of bioactive compounds in mycorrhizal species (Figure 5). Within the *Passifloraceae* family, only *Passiflora* species have been evaluated, mainly the leaves of *P. alata* (Oliveira *et al.*, 2015a,b; Muniz *et al.*, 2021;2022a), *Passiflora edulis* f. *flavicarpa* Deg. (Oliveira *et al.*, 2019a;2020), *Passiflora cincinnata* Mast. (Falcão; Silva, 2022), and *Passiflora setacea* DC. (Muniz *et al.*, 2022b) and some of these species are used in the anxiolytic herbal medicine industry (Fonseca *et al.*, 2020; Oliveira *et al.*, 2020).

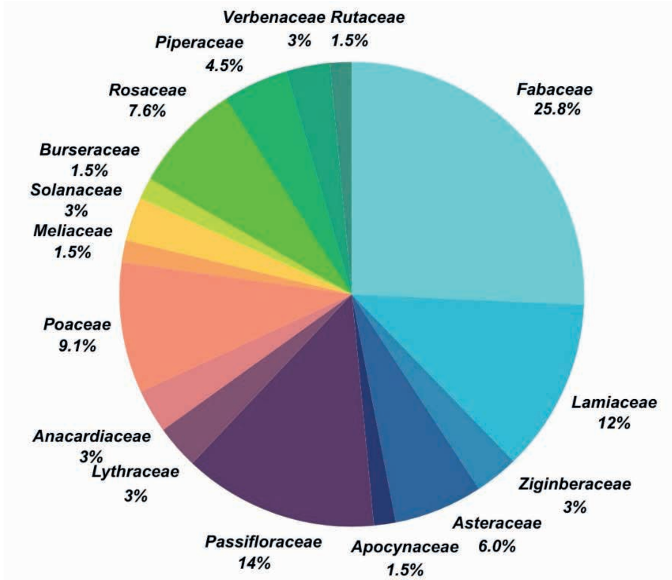


Figure 5. Botanical families studied in Brazil using arbuscular mycorrhizal fungi (AMF) to increase the production of phytochemicals. Number of experimental studies= 66 (Freitas *et al.,* 2004a,b; Andrade *et al.*, 2010;2013; Oliveira *et al.*, 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al.*, 2013;2018; Riter Netto *et al.*, 2014; Silva *et al.*, 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al.*, 2015;2023; Lima *et al.*, 2015a,b;2017; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Santos *et al.*, 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al.*, 2018;2020; Silva; Maia, 2018; Chiomento *et al.*, 2019;2021;2022; Cordeiro *et al.*, 2019; Cruz *et al.*, 2019;2020; Ferrari *et al.*, 2020; Merlin *et al.*, 2020; Vieira *et al.*, 2021; Trindade *et al.*, 2021; Muniz *et al.*, 2021;2022a,b;2023; Marcolino *et al.*, 2021; Falcão; Silva, 2022; Falcão *et al.*, 2022;2023b;2024b; Palhares Neto *et al.*, 2022; Pinc *et al.*, 2022; Souza *et al.*, 2022; Luz *et al.*, 2023; Nardi *et al.*, 2024; Melato *et al.,* 2024).

From the studies on terpene production in mycorrhizal *Lamiaceae*, three of them evaluated *Mentha* species (Freitas *et al.,* 2004b; Silva *et al.,* 2014b; Urcoviche *et al.,* 2015), two of them studied *Ocimum basilicum* L. (Morelli *et al.,* 2017; Silva *et al.,* 2021b), in addition to assays using *Salvia officinalis* L. (Cruz *et al.,* 2019), *Plectranthus amboinicus* (Lour.) Spreng (Merlin *et al.,* 2020), and *Melissa officinalis* L. (Pinc *et al.*, 2022). These studies are relevant, considering that essential oils have potential in the food industry due to their antimicrobial and antioxidant properties (Inanoglu *et al.,* 2023).

It was expected that the most evaluated legumes would be those of food and economic importance, nevertheless, the most studied were those of ethnobotanical relevance, such as *Libidibia ferrea* (Mart. ex Tul.) L. P. Queiroz, *Anadenanthera colubrina* (Vell.) Brenan, *Inga vera* Willd., and *Hymenaea martiana* Hayne (Pedone Bonfim *et al.*, 2013; Lima *et al.*, 2015; Silva *et al.*, 2014a,b;2018a;2021a; Santos *et al.*, 2017;2020;2021b; Falcão *et al.*, 2022;2023b;2024b; Muniz *et al.*, 2023). In addition, all experiments on the phytochemistry of mycorrhizal legumes were conducted in the Northeast of Brazil, which hosts over 1179 species from this plant family (Flora e Funga do Brasil, 2024).

The most studied plant parts were the leaves alone and the aerial part (Figure 6a), with the inflorescence being one of the least studied organs (1.4% of the studies). The more significant number of studies on leaves likely reflects the potential of this organ to produce and present an optimized anabolism due to mycorrhizal inoculation, which could make up herbal medicines. Although Brazilian studies on the phytochemistry of mycorrhizal species represent approximately 10% of the research in this area of mycorrhizology, there is a need to validate the benefits reported in greenhouses under field conditions (Figure 6b). Thus, only 9.1% of the studies have been conducted in experimental fields (Cordeiro *et al.*, 2019), especially for *L. ferrea* (Silva *et al.*, 2018a; Santos *et al.*, 2017;2020;2021b). This reflects the need to plan studies that consider field conditions to develop protocols that can be reproduced in cultivation sites established by companies that manufacture and market phytoformulations.

To assess the mycorrhizal efficiency in the production of secondary metabolites, compounds from phenolic origin were estimated in more than 55% of the studies, followed by the terpene group (39.8%) (Figure 7a). However, alkaloids, which are extremely important in chemotherapy treatments (Dhyani *et al.*, 2022), were only quantified in the studies by Andrade *et al.* (2013) and Luz *et al.* (2023), confirming the need for more research into this compound group, which are barely addressed from a mycorrhizal perspective.

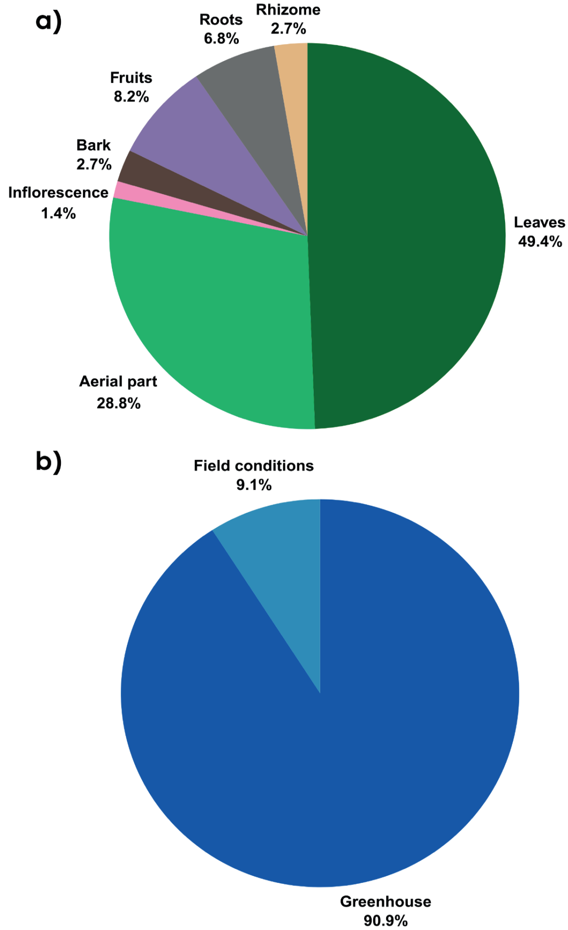


Figure 6. a) Plant parts used to assess bioactive compounds in mycorrhizal species. b) Sites where the experiments were conducted to quantify the phytochemistry of mycorrhizal species in studies developed in Brazil. Number of experimental studies= 66 (Freitas *et al.,* 2004a,b; Andrade *et al.*, 2010;2013; Oliveira *et al.*, 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al.*, 2013;2018; Riter Netto *et al.*, 2014; Silva *et al.*, 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al.*, 2015;2023; Lima *et al.*, 2015a,b;2017; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Santos *et al.*, 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al.*, 2018;2020; Silva; Maia, 2018; Chiomento *et al.*, 2019;2021;2022; Cordeiro *et al.*, 2019; Cruz *et al.*, 2019;2020; Ferrari *et al.*, 2020; Merlin *et al.*, 2020; Vieira *et al.*, 2021; Trindade *et al.*, 2021; Muniz *et al.*, 2021;2022a,b;2023; Marcolino *et al.*, 2021; Falcão; Silva, 2022; Falcão *et al.*, 2022;2023b;2024b; Palhares Neto *et al.*, 2022; Pinc *et al.*, 2022; Souza *et al.*, 2022; Luz *et al.*, 2023; Nardi *et al.*, 2024; Melato *et al.,* 2024).

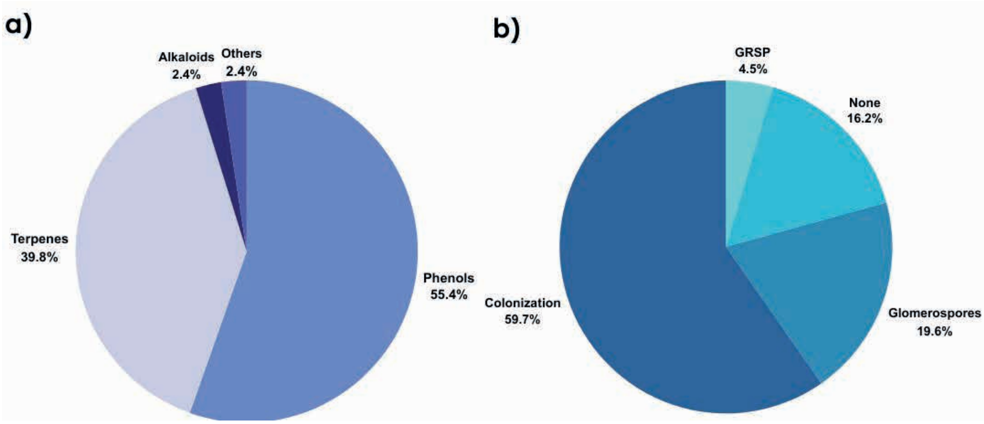


Figure 7. a) Compounds evaluated in phytochemical studies and b) parameters used to evaluate mycorrhizal activity in plants inoculated with arbuscular mycorrhizal fungi (AMF) based on studies conducted in Brazil. GRSP= Glomalin-Related Soil Proteins; Glomerospores= Glomerospore production; Colonization= Colonization percentage. Number of experimental studies= 66 (Freitas *et al.,* 2004a,b; Andrade *et al.,* 2010;2013; Oliveira *et al*., 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al*., 2013;2018; Riter Netto *et al*., 2014; Silva *et al*., 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al*., 2015;2023; Lima *et al*., 2015a,b;2017; Urcoviche *et al*., 2015; Morelli *et al*., 2017; Santos *et al*., 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al*., 2018;2020; Silva; Maia, 2018; Chiomento *et al*., 2019;2021;2022; Cordeiro *et al*., 2019; Cruz *et al*., 2019;2020; Ferrari *et al*., 2020; Merlin *et al*., 2020; Vieira *et al*., 2021; Trindade *et al*., 2021; Muniz *et al*., 2021;2022a,b;2023; Marcolino *et al*., 2021; Falcão; Silva, 2022; Falcão *et al*., 2022;2023b;2024b; Palhares Neto *et al*., 2022; Pinc *et al*., 2022; Souza *et al*., 2022; Luz *et al*., 2023; Nardi *et al*., 2024; Melato *et al.,* 2024).

In studies on mycorrhizal benefits in the production of plant bioactive compounds, the most common method used to assess the presence of the fungus in the root was to estimate mycorrhizal colonization using the methods of Giovannetti; Mosse (1980) and McGonigle *et al.* (1990). However, around 16% of the studies did not investigate mycorrhizal parameters, which is a concern because many of the explanations for how AMF can enhance the synthesis of bioactive compounds have been attributed to mycorrhizal activity in roots (Oliveira *et al*., 2015a) and rhizosphere (Hristozkova *et al*., 2017; Falcão *et al*., 2023b).

Another important aspect of academic productivity is the establishment of partnerships between research groups from different countries (Rostan; Ceravolo, 2015), which seems to be a limitation for most Brazilian groups in the field of mycorrhizal plant phytochemistry that often do not have a national and/or international network. In any case, the number of papers with international partnerships is on the rise, as seen in the papers by Trindade *et al.* (2021), Oliveira *et al.* (2022), Falcão *et al.* (2023b; 2024b), Muniz *et al.* (2023), Wu *et al.* (2023), Luz *et al.* (2023), Nardi *et al.* (2024), and Falcão *et al.* (2024a,b), which had the collaboration of researchers from universities in the United States, Canada, China, India, and Spain.

Based on the continental dimensions of Brazil, data are presented on the publication of papers on the phytochemistry of mycorrhizal species in the five geographical regions of this country. Thus, these results will be presented in the next chapters, considering the overview of the evaluated studies.

4. CONCLUSION AND PERSPECTIVES

Mycorrhizal biotechnology is an essential tool that can help to obtain a high-quality plant material that can be used to produce food, cosmetics, and medicines. Currently, several studies on the phytochemistry of mycorrhizal species have been conducted in Brazil, mainly in the Northeast (Figure 8); however, the recommendation for use must still be evaluated with caution, given the factors that can regulate the AMF efficiency, including climate, soil characteristics, and symbiotic partners. Given this, the lack of studies in the Central-West region, from a phytochemical perspective, needs to be encouraged, as this location has other climatic characteristics.

Given that the focus of research has been on phenolics and terpenes, it is important to fill the gap to understand how the production of Nitrogen compounds occurs, which have been poorly evaluated. In addition, it is necessary to explore the varied species of AMF occurring and isolated in the country, whose relationships with some plants are not yet known and could provide advantageous information to increase the production of biomolecules of industrial interest.

In addition, it is essential to develop new field studies aimed not only at improving the synthesis of molecules but also at understanding the mechanisms involved, the relationships established by the soil microbiota, and the ideal conditions for plant production, thus enabling the development of specific protocols that can meet the need of farmers and large industries.

This review aimed to compile the various aspects covered in studies of the phytochemistry of mycorrhizal species in Brazil and thus serve as an incentive for the creation of new research groups distributed throughout the country, which will help to clarify the role of mycorrhizal symbiosis in improving the plant biomass used in various industry sections.

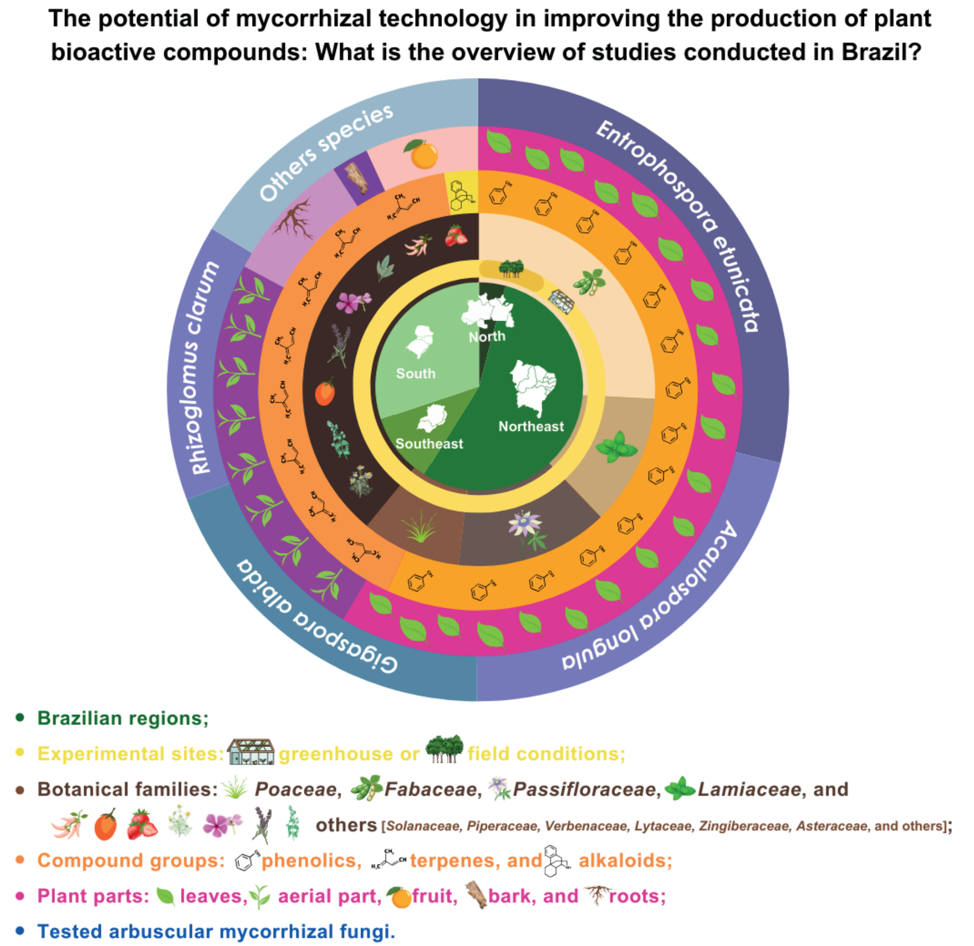


Figure 8. Overview of mycorrhizal species phytochemistry studies in Brazil (Freitas *et al.,* 2004a,b; Andrade *et al*., 2010;2013; Oliveira *et al*., 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al*., 2013;2018; Riter Netto *et al*., 2014; Silva *et al*., 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al*., 2015;2023; Lima *et al*., 2015a,b;2017; Urcoviche *et al*., 2015; Morelli *et al*., 2017; Santos *et al*., 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al*., 2018;2020; Silva; Maia, 2018; Chiomento *et al*., 2019;2021;2022; Cordeiro *et al*., 2019; Cruz *et al*., 2019;2020; Ferrari *et al*., 2020; Merlin *et al*., 2020; Vieira *et al*., 2021; Trindade *et al*., 2021; Muniz *et al*., 2021;2022a,b;2023; Marcolino *et al*., 2021; Falcão; Silva, 2022; Falcão *et al*., 2022;2023b;2024b; Palhares Neto *et al*., 2022; Pinc *et al*., 2022; Souza *et al*., 2022; Luz *et al*., 2023; Nardi *et al*., 2024; Melato *et al.,* 2024).
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CHAPTER 2

PHYTOCHEMISTRY OF MYCORRHIZAL SPECIES: AN APPROACH TO RESEARCH CONDUCTED IN THE BRAZILIAN NORTHEAST

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ABSTRACT: The Brazilian Northeast is the region with the largest number of studies evaluating the bioactive compound production in plants associated with arbuscular mycorrhizal fungi (AMF), especially in *Pernambuco*. Thus, the most studied botanical families were *Fabaceae* and *Passifloraceae*, highlighting species native to the *Caatinga* biome, which are of medicinal interest, such as *Libidibia ferrea* (Mart. ex Tul.) L. P. Queiroz and *Mimosa tenuiflora* (Willd.) Poir, as well as some cultivated species, including *Passiflora edulis* f. *flavicarpa* Deg. and *Zea mays* L. Mycorrhizal technology was also effective in increasing the production of compounds in other plants, such as *Mentha* x *piperita* L. and *Punica granatum* L., confirming its potential for enhancing the synthesis of plant bioactive compounds. The most

used mycorrhizal isolates were *Acaulospora longula* Spain & N.C. Schenck and *Gigaspora albida* Schenck & G.S. Sm. The studies mainly reported the influence of AMF in improving the biosynthesis of foliar bioactive compounds to add value to this organ that is often thrown away. The Northeast region of Brazil is a reference in research into the potential use of AMF to optimize the production of plant bioactive metabolites of interest to the pharmaceutical, cosmetic, and nutraceutical industries.
KEYWORDS: *Acaulospora*, bioactive compounds, *Fabaceae*.

1. INTRODUCTION

The Northeast region of Brazil covers 18% of the national territory, occupying around 1,551,991 km^2, and includes the states of Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, and Bahia (IBGE, 2019). The climate in this region is characterized by high temperatures, with averages that can reach 30 ºC (INMET, 2024). The variable vegetation is due to the occurrence of *Caatinga*, Atlantic Forest, and *Cerrado* biomes, which occur in the region, in addition to sandbanks and mangroves along the coast (IBGE, 2019).

Moreover, the cultivation of food plants is significant, especially maize, soybeans, sugarcane, cocoa, coffee, tropical fruits (grapes, bananas, mangoes, pineapples, papaya, melons, watermelons, among others), and cassava, which plays a fundamental role in the region's economy (IBGE, 2022).

An approach for growing plants and improving crop production of metabolites is by ameliorating soil factors using mycorrhizal technology (Falcão *et al*., 2024a). Thus, around 50% of Brazilian phytochemical studies on plants associated with AMF have been carried out in the Northeast region, mainly in Pernambuco (Figure 1). Most of these studies were conducted by the research group on Fungi of Agricultural Importance, registered in the *Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)* and comprising the Laboratory for Analysis, Research and Studies on Mycorrhizae (LAPEM) and the Laboratory for Mycorrhizal Technology (LTM), both at the University of Pernambuco, representing about 49% of Brazilian research in this field.

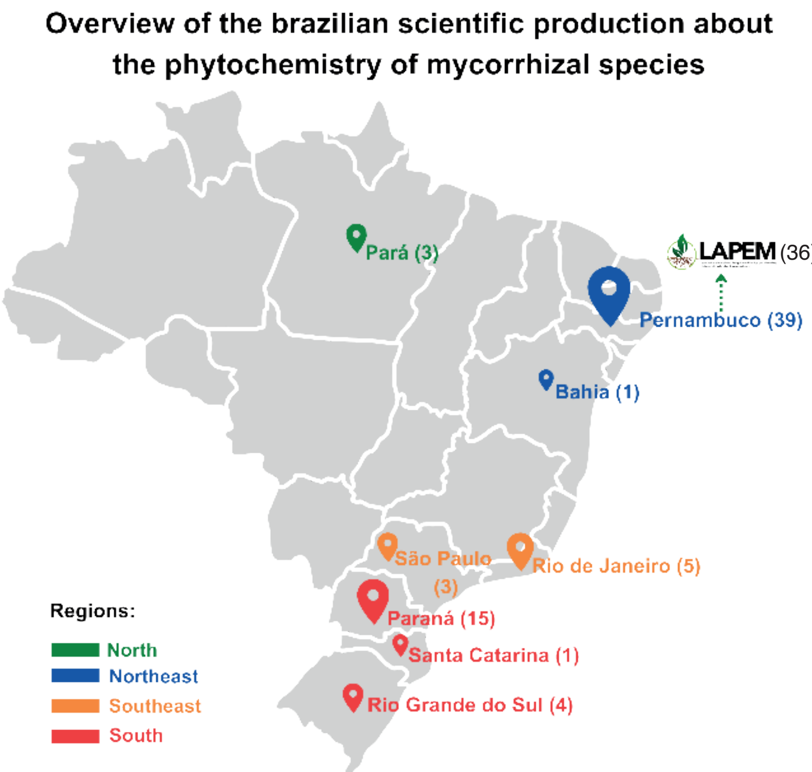


Figure 1. Overview of Brazilian papers on the phytochemistry of mycorrhizal species (Freitas *et al.,* 2004a,b; Andrade *et al*., 2010;2013; Oliveira *et al*., 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al*., 2013;2018; Riter Netto *et al*., 2014; Silva *et al*., 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al*., 2015;2023; Lima *et al*., 2015a,b;2017; Urcoviche *et al*., 2015; Morelli *et al*., 2017; Santos *et al*., 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al*., 2018;2020; Silva; Maia, 2018; Chiomento *et al*., 2019;2021;2022; Cordeiro *et al*., 2019; Cruz *et al*., 2019;2020; Ferrari *et al*., 2020; Merlin *et al*., 2020; Vieira *et al*., 2021; Trindade *et al*., 2021; Muniz *et al*., 2021;2022a,b;2023; Marcolino *et al*., 2021; Falcão; Silva, 2022; Falcão *et al*., 2022;2023b;2024b; Palhares Neto *et al*., 2022; Pinc *et al*., 2022; Souza *et al*., 2022; Luz *et al*., 2023; Nardi *et al*., 2024; Melato *et al.,* 2024).
LAPEM: Laboratório de Análises, Pesquisas e Estudos em Micorrizas da Universidade de Pernambuco

This integrative review aimed to compile phytochemical studies conducted in Northeast Brazil that used mycorrhizal species. To this end, the search for papers was the same as in the first chapter.

2. RESULTS AND DISCUSSION: REGIONAL EVALUATION OF PHYTOCHEMICAL STUDIES ON MYCORRHIZAL SPECIES

Among the studies that have evaluated the phytochemistry of plants associated with AMF in Northeast Brazil, approximately 47% and 23% of the studies investigated species from the *Fabaceae* and *Passifloraceae*, respectively (Pedone Bonfim *et al.,* 2013; Silva *et al.,* 2014a,b;2018a;2021a; Lima *et al.,* 2015a; Oliveira *et al.,* 2015a,b,c;2019;2020; Santos

et al., 2017;2020;2021a; Silva; Silva, 2017; Muniz *et al.,* 2021;2022a,b;2023; Falcão *et al.,* 2022; 2023a;2024b; Falcão; Silva, 2022), studies with *Anacardiaceae* (Oliveira *et al.,* 2013; Silva; Maia, 2018), *Lythraceae* (Silva *et al.,* 2014d; Silva; Silva, 2020), *Burseraceae* (Lima *et al.,* 2017), *Poaceae* (Silva *et al.,* 2019), *Myrtaceae* (Marcolino *et al.,* 2021), *Verbenaceae* (Palhares Neto *et al.,* 2022), and *Lamiaceae* (Silva *et al.,* 2014c) were also developed, showing a considerable diversity of studied taxa, with numbers higher than those recorded in other Brazilian regions (Figure 2).

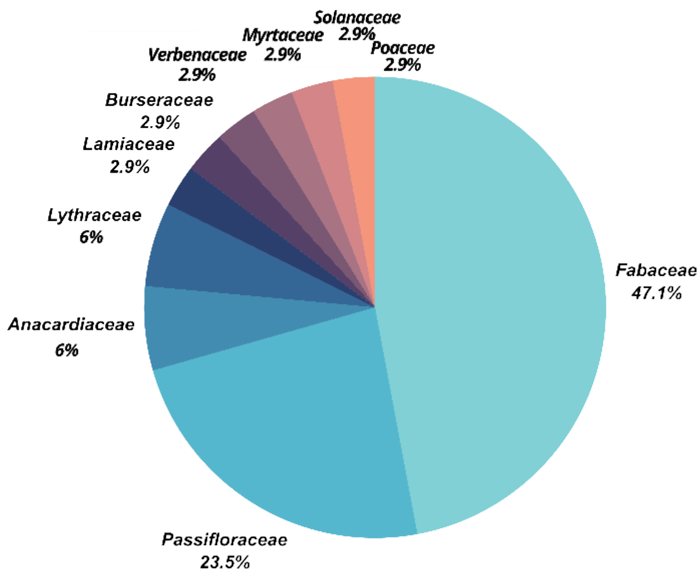


Figure 2. Botanical families studied in Northeast Brazil to evaluate the phytochemistry of mycorrhizal representatives (Pedone Bonfim *et al.,* 2013;2018; Oliveira *et al.,* 2013;2015a,b,c;2019a;2020; Silva *et al.,* 2014a,b,c,d;2018;2019;2021a; Lima *et al.,* 2015a;2017; Santos *et al.,* 2017;2020;2021a,b; Silva; Silva, 2017;2020; Silva; Maia, 2018; Muniz *et al.,* 2021;2022a,b;2023; Falcão; Silva 2022; Falcão *et al.*, 2022;2023a;2024b; Luz *et al.,* 2023).

Among the tree species that occur in the *Caatinga* and whose anabolism is enhanced by mycorrhizal technology, *L. ferrea*, *Commiphora leptophloeos* (Mart.) J.B. Gillett, and *Mimosa tenuiflora* (Wild.) Poir. stand out, as they are used in folk medicine to treat ailments, such as inflammation, flu, and respiratory problems (Albuquerque *et al.,* 2007; Albergaria *et al.,* 2019). Moreover, some are significant because they have a high relative importance index (RI), as the case with *Myracrodruon urundeuva* Allemao, *Amburana cearensis* Allemao, *H. martiana* and *A. colubrina*, due to the broad therapeutic spectrum of preparations using these plants (Albuquerque *et al.,* 2007). Data on mycorrhizal efficiency in trees can encourage the establishment of sustainable crops and help reduce the unplanned extractive use of organs from these species.

Among the studies, conducted in Brazil, on the metabolism of mycorrhizal plants, *A. longula, E. etunicata,* and *G. albida* stood out as the most used in research carried out in the Northeast region (Figure 3). These AMF are found naturally in *Caatinga* soils (Pontes *et*

al., 2017), however, they have different colonization strategies: members of *Gigasporaceae* have spores as their only reproductive structure and produce more mycelium in the soil than in the roots; the opposite is observed in taxa of *Acaulosporaceae* and *Entrophosporaceae*, which also propagate from hyphae fragments, with more expressive intraradicular colonization (Hart; Reader, 2002). Additionally, *E. etunicata* can adapt to different soil conditions (Weissenhorn *et al.,* 1994; Dashtebani *et al.,* 2014), perhaps reflecting the fungus choice in the research conducted in the country (Figure 3, Chapter 1).

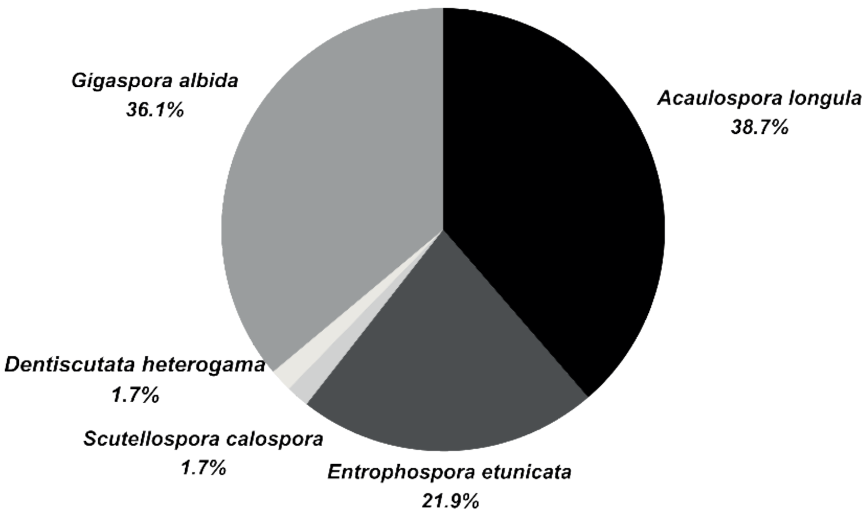


Figure 3. Tested mycorrhizal fungi species in phytochemical studies that used mycorrhizal plants in the Northeast region. *Acaulospora longula* Spain & N.C. Schenck, *Dentiscutata heterogama* (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl, *Entrophospora claroidea* (N.C. Schenck & G.S. Sm.) Błaszk., *Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno, *Gigaspora albida* N.C. Schenck & G.S. Sm. e *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders (Pedone Bonfim *et al*., 2013;2018; Oliveira *et al*., 2013;2015a,b,c;2019;2020; Silva *et al*., 2014a,b,c,d;2018a;2019a;2021; Lima *et al*., 2015a;2017; Santos *et al*., 2017;2020;2021a,b; Silva; Silva, 2017;2020; Silva; Maia, 2018; Muniz *et al*., 2021;2022a,b;2023; Falcão; Silva, 2022; Falcão *et al*., 2022;2023a;2024b; Luz *et al*., 2023).

The anabolic products of *Fabaceae* trees, in response to mycorrhization, were quantified in *A. cearensis* (Oliveira *et al.,* 2015c), *A. colubrina* (Pedone Bonfim *et al.,* 2013; Falcão *et al.,* 2022;2023a;2024b), *I. vera* (Lima *et al.,* 2015a), *L. ferrea* (Silva *et al.,* 2014a,b; 2018a; 2021a; Santos *et al.,* 2017; 2020; 2021a) and *M. tenuiflora* (Silva; Silva, 2017; Pedone Bonfim *et al.,* 2018). The majority evaluated the foliar phytochemistry (Silva *et al.,* 2014c,d; Lima *et al.,* 2015a; Oliveira *et al.,* 2015c; Silva; Silva, 2017; Pedone Bonfim *et al.,* 2018; Muniz *et al.,* 2021;2022a,b;2023; Falcão *et al.,* 2022;2023a;2024b), with one study using the entire aerial part (Pedone Bonfim *et al.,* 2013) and, of these, only Silva *et al.* (2014d) used leaves obtained from a field experiment. In contrast, the bark of the stem (Santos *et al.,* 2017; Silva *et al.,* 2018) and fruits were the least studied organs (Santos *et al.,* 2020;2021b) (Figure 4).

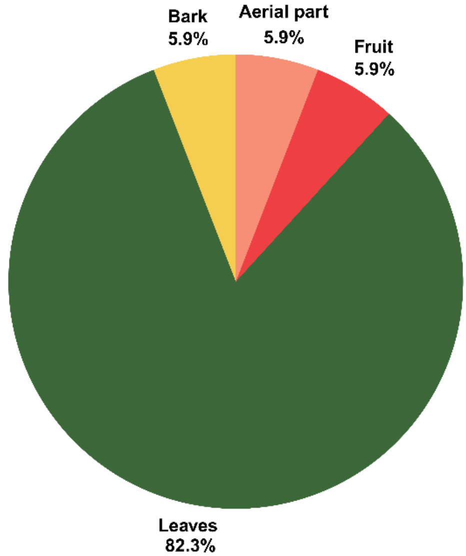


Figure 4. Plant parts used to evaluate the phytochemistry in research conducted in the Northeast using mycorrhizal plants (Pedone Bonfim *et al.*, 2013;2018; Oliveira *et al.*, 2013;2015a,b,c;2019a;2020; Silva *et al.*, 2014a,b,c,d;2018a;2019;2021; Lima *et al.*, 2015a;2017; Santos *et al.*, 2017;2020;2021a,b; Silva; Silva, 2017;2020; Silva; Maia, 2018; Muniz *et al.*, 2021;2022a,b;2023; Falcão *et al.*, 2022;2023a;2024b; Luz *et al.*, 2023).

Soils from *Caatinga* areas have been used in most studies applying AMF to enhance the secondary anabolism of *Fabaceae* (Pedone Bonfim *et al.*, 2013; 2018; Lima *et al.*, 2015a; 2017; Oliveira *et al.*, 2015c; Santos *et al.*, 2017; 2020; 2021b; Silva *et al.*, 2018a; 2021a; Falcão *et al.*, 2022; 2023a; 2024b; Muniz *et al.*, 2023); this substrate is poor (4 to 12.68 mg P dm^{-3}), and because of this, some studies with this family have also investigated the effects of phosphate fertilization (Pedone Bonfim *et al.*, 2013; Silva *et al.*, 2021a; Falcão *et al.*, 2024b), or organic substrates (Muniz *et al.*, 2023) associated with the use of AMF to optimize the biosynthesis of secondary metabolites.

Another plant group that has been widely studied is the genus *Passiflora*. Passion fruits are included in the Brazilian Pharmacopoeia (ANVISA, 2019) and the *Relação Nacional de Plantas Medicinais de Interesse ao Sistema Único de Saúde* (*RENISUS*) (Brasil, 2009) and are commonly used in folk medicine and by the pharmaceutical industry in the preparation of calming and sedative formulations (Klein *et al.,* 2014). The leaves were the only plant organ evaluated in mycorrhizal *Passiflora*, aligning with the Brazilian Pharmacopoeia, which cites this organ as a medically active part of these plants and as the part used to produce herbal medicines (ANVISA, 2019).

In this context, positive results of mycorrhization on foliar metabolism were reported in eight studies with four passion fruit species (*Passiflora alata* Curtis*, Passiflora cincinnata* Mast.*, P. edulis* f. *flavicarpa* Deg.*,* and *Passiflora setacea* DC.) (Oliveira *et al.*,

2015a,b;2019;2020; Muniz *et al.*, 2021;2022a,b; Falcão; Silva, 2022). Most of them used soil collected in the *Caatinga*, with an acid pH (5.6 - 6.1), low phosphorus content (4.26 - 4.92 mg dm^{-3}, Mehlich), which was increased when fertilizers were applied (Oliveira *et al.*, 2015b,c;2019a;2020; Muniz *et al.*, 2022a,b;2023; Falcão; Silva, 2022). Therefore, it is possible to obtain phytomass from mycorrhizal passion fruit vines to integrate the production chain of anxiolytic herbal medicines, since *P. edulis* seedlings inoculated with *A. longula*, for example, had their foliar vitexin production enhanced, making it possible to produce up to 900 tablets with the extract obtained, which is 60% higher than the projected when using extracts from non-mycorrhizal plants (Oliveira *et al.*, 2019a).

All studies with representatives of *Passiflora* were carried out in a greenhouse, under uncontrolled environmental conditions of light and temperature, with a cultivation period varying between 61 and 134 days (Oliveira *et al.*, 2015a,b; 2019a; 2020; Muniz *et al.*, 2021; 2022a,b; Falcão; Silva, 2022). However, it is worth mentioning the importance of field studies to prove symbiotic efficiency in edaphic systems present in more than 45,000 hectares destined for passion fruit cultivation in Brazil (IBGE, 2022).

Other plant species also had their production of compounds favored by AMF inoculation, as was the case with *Mentha* x *piperita* L. var. *citrata* (Ehrh.) Briq. (*Lamiaceae*), and *Punica granatum* L. (*Lythraceae*), medicinal plants that showed an increase in the synthesis of linalool and phenolic compounds, respectively (Silva *et al.*, 2014c,d). The mycorrhizal technology has also favored total flavonoid concentration of *Zea mays* L. (*Poaceae*) leaves, increasing the nutraceutical quality related to flavonoid content (Silva *et al.,* 2019).

In summary, the Northeast is the region with the highest number of studies on the phytochemistry of mycorrhizal species in Brazil, with 40 published studies, 34 of which were experimental. These papers evaluated the influence of five AMF species, with representatives of ten botanical families, in enhancing the synthesis of bioactive compounds, especially foliar phenolics, followed by terpenes and alkaloids. A smaller number of studies have evaluated this mycorrhizal benefit in the bark, fruit, and aerial part, while studies using roots are rare. Details of these research studies are described in Table 1.

Table 1. Phytochemical experimental research conducted in Northeast Brazil using mycorrhizal species

Plant species	Plant part	Evaluated compound group	AMF species	Evaluated mycorrhizal parameters	Reference
Myracrodruon urundeuva M. Allemão	Aerial part	Phenols	*Acaulospora longula* Spain & N.C. Schenck; *Gigaspora albida* N.C. Schenck & G.S. Sm.	Mycorrhizal colonization	Oliveira *et al.* (2013)
Anadenanthera colubrina (Vell.) Brenan	Aerial part	Phenols	*A. longula*; *G. albida*	None	Pedone Bonfim *et al.* (2013)
Punica granatum L.	Leaves	Phenols	*A. longula*; *G. albida*	Mycorrhizal colonization; Glomerospores	Silva *et al.* (2014a)
Mentha × piperita L.	Leaves	Terpenes	*Rhizoglomus clarum* (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl; *A. longula*; *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	Mycorrhizal colonization	Silva *et al.* (2014b)
Libidibia ferrea (Mart. ex Tul.) L.P. Queiroz	Leaves	Phenols	*A. longula*; *G. albida; Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno	Mycorrhizal colonization	Silva *et al.* (2014c)
L. ferrea	Leaves	Phenols	*A. longula*; *G. albida; E. etunicata*	Mycorrhizal colonization; Glomerospores	Silva *et al.* (2014d)
Inga vera Willd.	Leaves	Phenols	*A. longula*; *G. albida; E. etunicata*	Mycorrhizal colonization	Lima *et al.* (2015)
Amburana cearensis (Allemão) A.C.Sm.	Leaves	Phenols	*A. longula*; *G. albida; E. etunicata*	Mycorrhizal colonization	Oliveira *et al.* (2015a)
Passiflora alata Curtis	Leaves	Phenols	*G. albida*	Mycorrhizal colonization	Oliveira *et al.* (2015b)
P. alata	Leaves	Phenols	*G. albida*	Mycorrhizal colonization	Oliveira *et al.* (2015c)
Commiphora leptophloeos (Mart.) J.B. Gillett	Leaves	Phenols	*A. longula*; *G. albida; E. etunicata*	Mycorrhizal colonization	Lima *et al.* (2017)
L. ferrea	Bark	Phenols	*A. longula*; *G. albida; E. etunicata*	Mycorrhizal colonization; Glomerospores	Santos *et al.* (2017)
Mimosa tenuiflora (Willd.) Poir.	Leaves	Phenols	*A. longula*; *G. albida; E. etunicata*	Mycorrhizal colonization	Silva; Silva (2017)
M. tenuiflora	Leaves	Phenols	*G. albida; E. etunicata*	Mycorrhizal colonization; Glomerospores	Pedone Bonfim *et al.* (2018)
M. urundeuva	Leaves	Phenols	*A. longula*; *G. albida*	None	Silva; Maia (2018)
L. ferrea	Bark	Phenols	*A. longula*; *G. albida; E. etunicata*	None	Silva *et al.* (2018)

Passiflora edulis f. *flavicarpa* Deg.	Leaves	Phenols; Terpenes	*A. longula*; *G. albida; E. etunicata*	Mycorrhizal colonization	Oliveira *et al.* (2019a)
Zea mays L.	Leaves	Phenols	*A. longula*; *G. albida; E. etunicata; Dentiscutata heterogama* (Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl	None	Silva *et al.* (2019)
P. edulis	Leaves	Phenols; Terpenes	*A. longula*	Mycorrhizal colonization; Glomerospores	Oliveira *et al.* (2020)
L. ferrea	Fruits	Phenols; Terpenes	*A. longula*; *G. albida; E. etunicata*	Mycorrhizal colonization	Santos *et al.* (2020)
P. granatum	Leaves	Phenols	*A. longula*; *E. etunicata*	Mycorrhizal colonization	Silva; Silva (2020)
Psidium guajava L.	Leaves	Phenols	*A. longula*	None	Marcolino *et al.* (2021)
P. alata	Leaves	Phenols; Terpenes	*A. longula*; *G. albida*	Mycorrhizal colonization; GRSP	Muniz *et al.* (2021)
L. ferrea	Fruits	Phenols	*A. longula*; *G. albida; E. etunicata*	None	Santos *et al.* (2021b)
L. ferrea	Leaves	Phenols	*A. longula*; *G. albida; E. etunicata*	Mycorrhizal colonization	Silva *et al.* (2021a)
A. colubrina	Leaves	Phenols	*A. longula*; *G. albida*	Mycorrhizal colonization; C-GRSP	Falcão *et al.* (2022)
Passiflora cincinnata Mast.	Leaves	Phenols; Terpenes	*A. longula*; *G. albida*	Mycorrhizal colonization; GRSP	Falcão; Silva (2022)
Passifora setacea DC.	Leaves	Phenols; Terpenes	*A. longula*; *G. albida*	Mycorrhizal colonization; GRSP	Muniz *et al.* (2022a)
P. alata	Leaves	Phenols; Terpenes	*A. longula*	Mycorrhizal colonization; Glomerospores	Muniz *et al.* (2022b)
Lippia alba (Mill.) N.E.Br. ex Britton & P. Wilson	Leaves	Terpenes	*E. etunicata; Fuscutata heterogama* Oehl, F.A. Souza, L.C. Maia & Sieverd.	Mycorrhizal colonization	Palhares Neto *et al.* (2022)
A. colubrina	Leaves	Phenols; Terpenes	*A. longula*; *G. albida*	Mycorrhizal colonization; GRSP	Falcão *et al.* (2023)
Hymenaea martiana Hayne	Leaves	Phenols	*A. longula*; *G. albida*; *E. etunicata*	Mycorrhizal colonization	Muniz *et al.* (2023)
Capsicum chinense Jacq.	Leaves	Phenols; Terpenes; Alkaloids	*A. longula*; *G. albida*; *E. etunicata*	Mycorrhizal colonization; Glomerospores	Luz *et al.* (2023)
A. colubrina	Leaves	Phenols; Terpenes	*A. longula*; *G. albida*	None	Falcão *et al.* (2024b)

GRSP: Glomalin-related soil proteins.

3. CONCLUSIONS

Northeast Brazil is an important hub for research into the phytochemistry of mycorrhizal plants, with a range of native and cultivated species. In this context, studies with the fungi *A. longula*, *G. albida,* and *E. etunicata* deserve to be highlighted, considering the benefits reported to plant anabolism. The cultivation of plants associated with AMF, mainly *Fabaceae* and *Passifloraceae*, which are found in national biomes, increased the production of phytomass with a higher yield of medicinal and cosmetic compounds. Therefore, mycorrhizal technology is a promising strategy for cultivating plants found in the Northeast region.

Furthermore, the relevance of research groups, equipped laboratories, and partnerships with specialists from various fields are crucial for the successful development of mycorrhizal protocols in the region.

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MYCORRHIZA APPLICATION TO ENHANCE THE PRODUCTION OF PLANT BIOACTIVE COMPOUNDS: A REVIEW OF STUDIES DEVELOPED IN THE BRAZILIAN SOUTH AND SOUTHEAST

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ABSTRACT: The South is the second largest region in terms of Brazilian studies conducted on the phytochemistry of mycorrhizal species. Along with the Southeast, most of the research focuses on evaluating the production of plant bioactive compounds in medicinal species. However, the studies on strawberry plants are relevant because they address the effect of mycorrhization on fleshy fruit quality

parameters and are the only ones to evaluate this plant organ in the region. In addition, most of the studies were conducted with *Cymbopogon citratus* [DC.] Stapf. and the mycorrhizal inocula most often tested were from representatives of *Entrophosporaceae*. Mycorrhizal colonization has been evaluated in most of the studies, nevertheless, data on the production of glomalin-related soil proteins and glomerospores remains relatively uncommon. In this regard, mycorrhizal technology has the potential to provide plant material from medicinal species with an outstanding accumulation of metabolites related to bioactive properties.
KEYWORDS: *Entrophospora*; essential oils, medicinal plants, secondary metabolites.

1. INTRODUCTION

The Southeast region of Brazil is the most populous and the richest in the country (IBGE, 2022a,b). The states of Espírito Santo, São Paulo, Minas Gerais, and Rio de Janeiro are mostly dominated by regions with a subtropical climate, with average annual temperatures of 20 to 23 ºC (Alvares *et al.*, 2013a,b). In agribusiness, sugarcane, tomatoes and coffee are the most economically important crops in the region (IBGE, 2022c). However, approaches using mycorrhizal technology, considering phytochemical studies, are mainly focused on the potential for optimizing the production of bioactive compounds in species of medicinal relevance.

The South of Brazil comprises the states of Paraná, Santa Catarina, and Rio Grande do Sul. Despite having the smallest territorial extension compared to other Brazilian regions, is economically important in the country (IBGE, 2022b). The fruit-growing market generates billions of *reais* per year, especially for apple, grape, and strawberry crops (Lima *et al.*, 2021; IBGE, 2022c). This condition is likely due to the mild climate, with average annual temperatures of 14 to 23 ºC (Alvares *et al.*, 2013a) and classified as subtropical (Alvares *et al.*, 2013b). In this context, arbuscular mycorrhizal fungi can be an alternative for enhancing phytochemical and fruit quality parameters (Chiomento *et al.,* 2021), an approach that has been carried out in some studies in this region.

2. RESULTS AND DISCUSSION: THE USE OF MYCORRHIZAL FUNGI TO INCREASE PHYTOCHEMICAL PRODUCTION IN SOUTHERN AND SOUTHEAST BRAZIL

The South has the second-highest number of Brazilian studies on the phytochemistry of plants associated with AMF. In this region, the most investigated plants were *Cymbopogon citratus* [DC.] Stapf. (*Poaceae*) (Lermen *et al.*, 2015; Cruz *et al.*, 2020; Silva *et al.*, 2021a,b; Souza *et al.*, 2022) and *Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier (*Rosaceae*) (Cordeiro *et al.*, 2019; Chiomento *et al.*, 2019;2021;2022; Nardi *et al.*, 2024).

Phytochemical studies of mycorrhizal plants conducted in this region have evaluated the effect of AMF on the production of metabolites in medicinal plants (Silva *et al.*, 2008; Lermen *et al.*, 2015; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Cruz *et al.*, 2019;2020;

Almeida *et al.*, 2020; Merlin *et al.*, 2020; Ferrari *et al.*, 2020; Silva *et al.*, 2021a,b; Silva *et al.*, 2021c; Pinc *et al.*, 2022; Souza *et al.*, 2022; Lermen *et al.*, 2023; Melato *et al.*, 2024) (Table 1), with the main focus on evaluating the biosynthesis of essential oils (Lermen *et al.*, 2015; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Cruz *et al.*, 2019;2020; Almeida *et al.*, 2020; Merlin *et al.*, 2020; Ferrari *et al.*, 2020; Silva *et al.*, 2021a,b; Silva *et al.*, 2021c; Pinc *et al.*, 2022; Souza *et al.*, 2022; Lermen *et al.*, 2023; Melato *et al.*, 2024) and oil-resin (Silva *et al.*, 2008).

In the south of the country, the main AMF isolates evaluated were *E. etunicata* and *R. clarum*, fungi that made up 61.90% of the experiments in this region (Lermen *et al.*, 2015; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Cruz *et al.*, 2019;2020; Merlin *et al.*, 2020; Ferrari *et al.*, 2020; Chiomento *et al.*, 2021;2022; Silva *et al.*, 2021a,b; Silva *et al.*, 2021c; Pinc *et al.*, 2022; Souza *et al.*, 2022; Lermen *et al.*, 2023; Melato *et al.*, 2024; Nardi *et al.*, 2024).

As in the other regions, most of the research was conducted in a greenhouse; however, only Cordeiro *et al.* (2019) evaluated the effect of mycorrhizal biostimulants under field conditions (Table 2). Leaves and other vegetative aerial parts were the most evaluated materials, accounting for around 85% of the research. Of these, only one study used the inflorescences to quantify metabolites (Almeida *et al.*, 2020).

The fruit was the second most studied plant organ (Cordeiro *et al.*, 2019; Chiomento 2019;2021; Nardi *et al.*, 2024), followed by the roots (Andrade *et al.*, 2013; Chiomento *et al.*, 2022; Nardi *et al.*, 2024) and the rhizome (Silva *et al.*, 2008; Ferrari *et al.*, 2020). Only four studies evaluated more than one plant organ at the same time (Andrade *et al.*, 2013; Ferrari *et al.*, 2020; Chiomento *et al.*, 2022; Nardi *et al.*, 2024), an approach that is relevant to understanding the physiology of mycorrhizal plants, which can be used in future studies.

Table 1. Overview of phytochemical studies on mycorrhizal plants conducted in the Brazilian South

Plant species	Plant part	Evaluated compound group	AMF species	Mycorrhizal parameters	References
Zingiber officinale Roscoe	Rhizome	Terpenes	*Dentiscutata heterogama* (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl; *Gigaspora decipiens* I.R. Hall & L.K. Abbott; *Acaulospora koskei* Blaszk.; *Acaulospora colombiana* (Spain & N.C. Schenck) Kaonongbua, J.B. Morton & Bever	Mycorrhizal colonization; Glomerospores	Silva *et al.* (2008)
Cymbopogon citratus [DC.] Stapf.	Aerial part	Terpenes	*Rhizoglomus clarum* (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl	None	Lermen *et al.* (2015)
Mentha crispa L.	Aerial part	Terpenes	*Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno; *R. clarum*	Mycorrhizal colonization; Glomerospores	Urcoviche *et al.* (2015)
Ocimum basilicum L.	Aerial part	Terpenes	*E. etunicata*	Mycorrhizal colonization; Glomerospores	Morelli *et al.* (2017)
Fragaria x *ananassa* (Duchesne ex Weston) Duchesne ex Rozier	Fruits	Phenols	AMF community	None	Chiomento *et al.* (2019)
Fragaria x *ananassa*	Fruits	Phenols	AMF community	Mycorrhizal colonization	Cordeiro *et al.* (2019)
Salvia officinalis L.	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Cruz *et al.* (2019)
Matricaria chamomilla L.	Inflorescence	Terpenes	AMF community	Mycorrhizal colonization	Almeida *et al.* (2020)
C. citratus	Aerial part	Phenols; Terpenes	*E. etunicata*; *R. clarum*	None	Cruz *et al.* (2020)
Curcuma longa L.	Rhizome and leaves	Phenols; Terpenes	*E. etunicata*; *R. clarum*	None	Ferrari *et al.* (2020)
Plectranthus amboinicus (Lour.) Spreng.	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Merlin *et al.* (2020)
Fragaria x *ananassa*	Fruits	Phenols	*E. etunicata;* AMF community	Mycorrhizal colonization	Chiomento *et al.* (2021)
O. basilicum	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Silva *et al.* (2021c)
C. citratus	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Silva *et al.* (2021b)
C. citratus	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization	Silva *et al.* (2021a)

Fragaria x *ananassa*	Leaves and roots	Phenols	*Entrophospora claroidea* (N.C. Schenck & G.S. Sm.) Błaszk.; *E. etunicata*; *Funneliformis geosporum* (T.H. Nicolson & Gerd.) C. Walker & A. Schluessler; *Diversispora versiformis* (P. Karst.) Oehl, G.A. Silva & Sieverd; *Glomus* sp.	Mycorrhizal colonization	Chiomento *et al.* (2022)
Melissa officinalis L.	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Pinc *et al.* (2022)
C. citratus	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Souza *et al.* (2022)
Lippia alba (Mill.) N.E.Br. ex Britton & P.Wilson	Aerial part	Terpenes	R. clarum	None	Lermen *et al.* (2023)
Fragaria × ananassa	Roots, aerial part, fruits, and crown	Phenols	*Acaulospora mellea* Spain & N.C. Schenck; *Acaulospora longula* Spain & N.C. Schenck; *Cetraspora pellucida* (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd.; *E. etunicata*; *Glomus* sp.; *Septoglomus viscosum* (T.H. Nicolson) C. Walker, D. Redecker, Stiller & A. Schüßler	Mycorrhizal colonization	Nardi *et al.* (2024)
Ruta graveolens L.	Aerial part	Terpenes	*R. clarum*; *E. etunicata*	Mycorrhizal colonization; Glomerospores	Melato *et al.* (2024)

AMF: arbuscular mycorrhizal fungi

In the Brazilian state, species from *Zingiberaceae* were also tested. *Zingiber officinale* Roscoe and *Curcuma longa* L., species listed in RENISUS (*Relação Nacional de Plantas de Interesse ao Sistema Único de Saúde*) (Brasil, 2009), produce around thrice more bioactive compounds when associated with selected AMF (Silva *et al.*, 2008; Ferrari *et al.*, 2020). Other plants of phytotherapeutic relevance evaluated by researchers in this region were *Lippia alba* (Mill.) N.E.Br. ex P. Wilson (*Verbenaceae*) (Lermen *et al.*, 2023), *Matricaria chamomilla* L. (*Asteraceae*) (Almeida *et al.*, 2020) and the *Lamiaceae*, *Mentha crispa* L. (Urcoviche *et al.*, 2015), *Ocimum basilicum* L. (Morelli *et al.*, 2017; Silva *et al.*, 2021c), *Salvia officinalis* L. (Cruz *et al.*, 2019), *Melissa officinalis* L. (Pinc *et al.*, 2022), and *Plectranthus amboinicus* (Lour.) Spreng. (Merlin *et al.*, 2020).

The studies conducted with strawberries reported greater accumulation of phenolic compounds (Cordeiro *et al.,* 2019; Chiomento *et al.,* 2019), something also reported in other organs (Chiomento *et al.,* 2022). These studies also differ from others due to the use of a greater number of mycorrhizal isolates (11 species) (Cordeiro *et al.*, 2019), as well as mixed inocula or those obtained from agricultural or forest sites (Chiomento *et al.,* 2019; 2021;2022). These, together with the study of Almeida *et al.* (2020), were the only ones that evaluated the effect of mycorrhizal communities in enhancing the production of bioactive compounds.

On the other hand, in the Southeast, different research groups have evaluated the potential of AMF to improve the concentration of compounds in phytomass, representing around 12% of national production. Species such as *Baccharis trimera* (Less.) DC., *Acmella oleracea* (L.) R.K. Jansen, *Mikania glomerata* Spreng., *Mikania laevigata* Sch. Bip. ex (*Asteraceae*) (Freitas *et al.*, 2004a; Vieira *et al.*, 2021; Almeida *et al.*, 2018), *Mentha arvensis* L. (*Lamiaceae*) (Freitas *et al.*, 2004b), *Canavalia ensiformis* (L.) D.C. (*Fabaceae*) (Andrade *et al.*, 2010), *Catharanthus roseus* (L.) G. Don (*Apocynaceae*), *Nicotiana tabacum* L. (*Solanaceae*) (Andrade *et al.*, 2013), *P. alata* (*Passifloraceae*) (Riter Netto *et al.*, 2014), and *Toona ciliata* M. Roem. (*Meliaceae*) were evaluated (Table 2).

Table 2. Studies conducted in the southeast Brazil that evaluated the effect of arbuscular mycorrhizal fungi (AMF) inoculation on phytochemistry

Plant species	Plant part	Evaluated compound group	AMF species	Mycorrhizal parameters	Reference
Baccharis trimera (Less.) DC.	Aerial part	Phenols	*Rhizoglomus clarum* (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl; *Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno; *Gigaspora margarita* W.N. Becker & I.R. Hall; *Acaulospora scrobiculata* Trappe	Mycorrhizal colonization	Freitas *et al.* (2004a)
Mentha arvensis L.	Aerial part	Terpenes	*R. clarum*; *E. etunicata*; *G. margarita*; *A. scrobiculata*	Mycorrhizal colonization	Freitas *et al.* (2004b)
Canavalia ensiformis (L.) DC.	Leaves	Phytochelatins	*E. etunicata*	Mycorrhizal colonization	Andrade *et al.* (2010)
Catharanthus roseus (L.) G.Don; *Nicotiana tabacum* L.	Leaves and roots	Alkaloids	*E. etunicata*; *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl	Mycorrhizal colonization	Andrade *et al.* (2013)
Passiflora alata Curtis	Aerial part	Phenols	*R. clarum*; *G. margarita*; *R. intraradices*; *E. etunicata*	Mycorrhizal colonization	Riter Netto *et al.* (2014)
Toona ciliata M. Roem.	Aerial part	Phenols	*R. clarum*; *G. margarita*; *E. etunicata*	Mycorrhizal colonization	Lima *et al.* (2015)
Mikania glomerata Spreng.; *Mikania laevigata Sch.Bip. ex Baker*	Leaves	Phenols; Terpenes	*Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl	Mycorrhizal colonization	Almeida *et al.* (2018)
Acmella oleracea (L.) R.K.Jansen	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Vieira *et al.* (2021)

In Rio de Janeiro, Freitas *et al.* (2004a) documented the enhanced production of phenols in *B. trimera*, a pioneer study in Brazil. This and other research conducted in the Southeast have evaluated the combined effects of mycorrhization and fertilization on the production of bioactive compounds in species with medicinal (Freitas *et al.*, 2004a,b; Riter Netto *et al.*, 2014; Vieira *et al.*, 2021), timber (Lima *et al.*, 2015), and food (Andrade *et al.*, 2010) applications.

In addition to this research, others with *Asteraceae* have been carried out (Veira *et al.*, 2021; Almeida *et al.*, 2018) and made it possible to select *R. clarum* and *E. etunicata* as biostimulants for phenol production (Vieira *et al.,* 2021). However, it is advisable to evaluate more AMF isolates, since in *guaco* species (*M. glomerata* and *M. laevigata*), which produce phenolics and are from the same botanical family, the inoculation of *Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl did not influence or reduce secondary anabolism, depending on the bioactive metabolite considered (Almeida *et al.,* 2018). Other inocula, with isolated AMF species and a mix of *R. clarum, E. etunicata, G. margarita*, and *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl, resulted in higher levels of metabolites when inorganic P was jointly applied to mycorrhizal plants (Freitas *et al.*, 2004a; Riter Netto *et al.*, 2014; Vieira *et al.*, 2021).

However, depending on the plant species and the group of secondary compounds, mycorrhization either dispensed fertilization supply (Freitas *et al.*, 2004a,b; Riter Netto *et al.*, 2014; Lima *et al.*, 2015; Vieira *et al.*, 2021) or was less efficient than chemical fertilizers (Andrade *et al.*, 2013) to optimize the biosynthesis of plant bioactive compounds. Although these results can be expected, the selection of adapted fungi to conditions of high fertility should be encouraged, considering that this condition is common in cultivated soils.

In the studies conducted in South and Southeast Brazil, mycorrhizal colonization was estimated in around 83% of the papers; of these, approximately 31% also quantified glomerospores; on the other hand, Glomalin-Related Soil Proteins (GRSP) production was not measured in soils from these experiments. As a result, there are still gaps regarding GRSP and glomerospore production in studies that aim to increase the content of bioactive compounds in plants associated with AMF in this region.

3. CONCLUSIONS AND PERSPECTIVES

The South and Southeast regions have crops of great economic importance for producing food plants. However, the main focus of studies involving the phytochemistry of mycorrhizal species has been to evaluate the production augmentation of bioactive compounds in medicinal species. An exception is the research on strawberry plants, which shows the efficiency of mycorrhizal technology in improving fruit quality parameters. Nevertheless, it is still necessary to establish efficient and well-characterized inocula for recommendation in different environmental conditions of this region.

Furthermore, research evaluating the effect of mycorrhization on the production of bioactive metabolites in maize and wheat crops, especially under field conditions, could favor cultivation protocols for some of the major agricultural products in the region.

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CHAPTER 4

OVERVIEW OF PHYTOCHEMICAL STUDIES ABOUT MYCORRHIZAL HOSTS IN THE BRAZILIAN NORTH AND CENTRAL-WEST REGIONS: CHALLENGES TO OVERCOME

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ABSTRACT: The North is one of the regions with the lowest number of studies dedicated to verifying the influence of arbuscular mycorrhizal fungi (AMF) on the production of biomolecules in plant tissues, second only to the Midwest. Pará is the only state to have studies from this perspective; these approaches were focused on *Piperaceae* representatives, which are recognized for their medicinal relevance. The studies in question used mycorrhizal isolates native to the northern region, aiming to evaluate their effect on reducing the impact caused by pathogens and verifying the leaf and root anabolism of mycorrhizal pepper grown in a greenhouse. Despite this, more research is needed, given the medicinal importance of species native to the northern region and the agricultural potential reported for the Midwest.

KEYWORDS: AMF, biomolecules, *Piperaceae*.

1. INTRODUCTION

The Brazilian North covers an area of approximately 3,850,593.104 km^2, the largest national region, equivalent to around 45% of the country's territory. Made up of seven states (IBGE, 2022), this region is marked by the Amazon Rainforest biome, one of the most exuberant and with the greatest fauna and flora biodiversity (Ministério do Meio Ambiente, 2024a). The equatorial climate prevails in this region and is characterized by high temperatures and humidity throughout the year (IBGE, 2024). These climatic conditions support the vast diversity that includes native plants with medicinal relevance (Breitbach *et al.*, 2013).

In addition to the Amazon Rainforest, some North areas are also home to the *Cerrado*, but it is in the Center-West where this biome is predominant (Ministério do Meio Ambiente, 2024b). This region covers approximately 19% of the national territory (IBGE, 2022), and is characterized by a tropical climate (IBGE, 2024). The Midwest is one of the country's main agricultural regions, contributing to the production of commodities (Ministério da Agricultura e Pecuária, 2022). Notwithstanding, only a few studies have investigated the role of inoculating arbuscular mycorrhizal fungi to optimize the synthesis of biomolecules responsible for pharmacological properties in plants native to the Amazon Rainforest. Additionally, there are no reports of this mycorrhizal benefit in plant species found in the Midwest, the region with the highest agricultural production in Brazil. Filling this gap is important, considering that species with medicinal potential from these regions, when harvested with higher levels of bioactive compounds, can be used in the pharmaceutical industry, adding value to other plant tissues that are not commonly used for therapeutic purposes.

This review aimed to gather studies on the phytochemistry of mycorrhizal plants carried out in North and Midwest Brazil. To this end, the search for papers was performed as described in the first chapter.

2. RESULTS AND DISCUSSION: SCENARIO OF STUDIES ON THE PHYTOCHEMISTRY OF MYCORRHIZAL SPECIES IN THE MAJOR BRAZILIAN REGIONS

In the North, the published papers about the benefits of mycorrhizal symbiosis in the biosynthesis of secondary biomolecules were for *Piperaceae*. The research is concentrated in the Pará state (Table 1) and was carried out by the Bioprospecting and Technological Innovation of Natural Products from the Amazon research group. The authors evaluated varied species of *Piper*, such as *Piper aduncum* L. (Oliveira *et al.*, 2019), *Piper nigrum* L. (Trindade *et al.*, 2021), and *Piper divaricatum* G. Mey (Oliveira *et al.*, 2022).

Table 1. Phytochemical studies about mycorrhizal plants conducted in the Brazilian North

Plant species	Plant part	Cultivation site	Evaluated bioactive compounds	AMF species	Mycorrhizal parameters	Reference
Piper aduncum L.	Leaves and roots	Greenhouse	Terpenes	*Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno; *Rhizoglomus clarum* (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl	Mycorrhizal colonization	Oliveira *et al.* (2019)
Piper nigrum L.	Leaves and roots	Greenhouse	Terpenes; Phenols	*E. etunicata*; *R. clarum*	None	Trindade *et al.* (2021)
Piper divaricatum G. Mey.	Leaves	Greenhouse	Terpenes; Phenols	*E. etunicata; R. clarum*	None	Oliveira *et al.* (2022)

Pepper species are relevant in medicine due to their anti-inflammatory, anti-dermatitis, antinociceptive (Branquinho *et al.*, 2017), antioxidant, and antimicrobial actions (Salleh *et al.*, 2014), conferring commercial value to the phytomass (Oliveira *et al.*, 2019b). The studies conducted with AMF aimed to reduce the impacts caused by *Neocosmospora piperis* (F.C. Albuq.) Sand.-Den. & Crous (previously known as *Fusarium solani* f. sp. *piperis* F.C. Albuq.) (Trindade *et al.*, 2021) and the influence of P in the substrate, considering different cultivation periods (Oliveira *et al.*, 2022), on the anabolism of essential oils and phenolics (Oliveira *et al.*, 2019b;2022; Trindade *et al.*, 2021).

All the studies conducted in this region were based on propagation by cuttings, in a greenhouse, using a commercial substrate, with plants inoculated with mixed inoculum. In these studies, isolates of *R. clarum* and *E. etunicata* (Oliveira *et al.*, 2019b;2022; Trindade *et al.*, 2021), native to Pará, were evaluated. This may be relevant for selecting inoculants adapted to the soil and climate conditions of the region.

Although Oliveira *et al.* (2019) verified mycorrhizal colonization in *P. aducum*, information on other aspects, such as the production of Glomalin-Related Soil Proteins (GRSP) and glomerospores were not reported in any of the studies conducted in the northern region. Therefore, data on mycorrhizal activity in the rhizosphere could be added to future studies conducted by researchers in Northern Brazil.

Despite being a region with agronomic relevance, there are no studies on the phytochemistry of mycorrhizal species conducted in the Central-West region; in this case, it is important to set up research groups in this field of mycorrhizology.

3. CONCLUSIONS AND PERSPECTIVES

The North has a wide biodiversity of medicinal plants, while the Center-West stands out for its agricultural relevance. However, little is known about the role of AMF in increasing the production of biomolecules in plants native to these regions. In context, it is necessary to conduct studies under field conditions to explore the therapeutic potential of mycorrhizal species and to understand the mechanisms involved in this interaction. Furthermore, it is important to investigate the effect of other mycorrhizal isolates present in these regions, considering that mycorrhizal efficiency can vary depending on the AMF species and host.

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Although the potential of mycorrhizal technology in the cultivation of plants of interest to the pharmaceutical, cosmetic and nutraceutical industries and the growing research in Brazil aimed at enhancing the production of plant bioactive compounds, more studies are needed to elucidate some of the mycorrhizal mechanisms involved in the anabolism of plants cultivated in Brazil. Furthermore, it is also necessary to understand the responsiveness of more commercially relevant hosts to different AMF species and isolates. To this end, more research must be carried out under field conditions to encourage the inclusion of mycorrhizal technology in the production chain of the plant-based cosmetics and herbal medicine industries.

The successful development of cultivation protocols using AMF, based on studies carried out in northeast Brazil, for example, highlights the biotechnological potential of mycorrhizal fungi to improve the anabolism of bioactive compounds that compose cosmetic and antiseptic products widely marketed in Brazil. However, considering Brazil's high botanical diversity, more studies need to be carried out to establish sustainable plant cultivation that comprises the portfolio of multinational cosmetics and phytomedicines companies. This strategy could help reduce the unplanned extraction of plant tissues.

Despite the various benefits recorded over more than two decades in Brazil, the recommendation to use mycorrhizal technology should not be generalized, as the AMF efficiency can be regulated by various soil and climate factors, in addition to the responses being modulated by the host and the mycorrhizal inocula tested. In this context, more mycorrhizal efficiency bioassays need to be conducted, especially in the North and Center-West regions, which have specific climatic characteristics and few reports on the effect of mycorrhizal symbionts on plant anabolism.

Finally, it is important to establish and strengthen national and international research networks to carry out cross-cutting and multidisciplinary proposals, which could help to elucidate new mechanisms of phytochemical modulation due to symbiont partners, in this case, representatives of *Glomeromycota*.

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